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Cholesterol Biosynthesis and Metabolism

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Summary. Cholesterol plays an essential role in cell membrane synthesis and in cell growth and differentiation. In mammalian cells, cholesterol can be synthesized from acetate precursors or taken up from dietary or exogenous sources. The major catabolic route for disposal of cholesterol involves conversion into excretable bile acids. The maintenance of cholesterol homeostasis is influenced and carefully controlled by multiple feedback mechanisms. The key regulatory targets of these feedback mechanisms are 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in cholesterol biosynthesis, the low-density lipoprotein (LDL) receptor in cholesterol uptake, and cholesterol 7α-hydroxylase in cholesterol catabolism. The elucidation of regulatory mechanisms in cholesterol metabolism has been greatly facilitated by the discovery of a new class of lipid-lowering drugs, the HMG-CoA reductase inhibitors. In addition to proving therapeutically useful in the treatment of hypercholesterolemia, these drugs have revealed novel regulatory steps in cholesterol metabolism and several new targets for future drug development. This manuscript reviews recent developments in the cholesterol biosynthetic pathway and the regulatory mechanisms that maintain cholesterol homeostasis.

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From a biochemical perspective, cholesterol is a necessary constituent for eukaryotic cell growth and development. The biosynthesis of cholesterol provides crucial building blocks for cell membranogenesis and membrane fluid regulation, and for the synthesis of sterol and nonsterol products that are important for normal cell function. Prevailing interest in cholesterol has revolved around its role in the development of atherosclerosis and cholelithiasis, and much research energy has been directed toward identifying mechanisms to decrease elevated serum cholesterol levels. More recent developments have underscored the essential nature of cholesterol in cell growth and development, and have begun to elucidate the mechanisms governing cholesterol homeostasis.

Cholesterol Metabolism

Cholesterol may be obtained for cellular metabolism either via uptake mediated by members of the low-density lipoprotein (LDL) receptor family or through biosynthesis. The uptake pathway involving the lipoprotein receptors may be further subdivided into an exogenous (dietary) pathway and an endogenous pathway (Figure 1). Although cholesterol uptake and biosynthesis are interdependent (i.e., changes in dietary cholesterol intake and cell requirements influence the rate of cholesterol biosynthesis through complex feedback mechanisms), cellular cholesterol requirements can be met equally well through either supply pathway.

Humans synthesize approximately 700–900 mg of cholesterol per day, while 300–500 mg are absorbed daily from dietary sources [2]. Normal daily cholesterol turnover is accounted for by excretion in the gastrointestinal tract (600 mg/day), conversion to bile acids (400 mg/day), sloughing skin (85 mg/day), biosynthesis of steroid hormones (50 mg/day), and by incorporation into membranes of actively dividing cells.

Most mammalian cells are capable of endogenous cholesterol synthesis [3]. In all species examined to date, cholesterol synthesis occurs primarily in four organs: the liver, gastrointestinal tract, skin, and "carcass" (i.e., striated muscle and bone marrow). With the exception of the liver, these organs demonstrate high rates of cell turnover and use large amounts of cholesterol for synthesizing new membranes. In humans, the liver and ileum are the primary sites of cholesterol biosynthesis [3]. Although the human liver has a low rate of cell turnover, it nevertheless synthesizes and takes up large amounts of cholesterol for lipoprotein, bile acid, and steroid hormone biosynthesis. Rates of hepatic cholesterol biosynthesis can be further increased during the extensive hepatocellular regeneration that occurs in patients who have undergone partial hepatectomy [4]. A diurnal variation in hepatic cholesterol synthesis (increased rate at night) has also been demonstrated in animals and humans [5,6].

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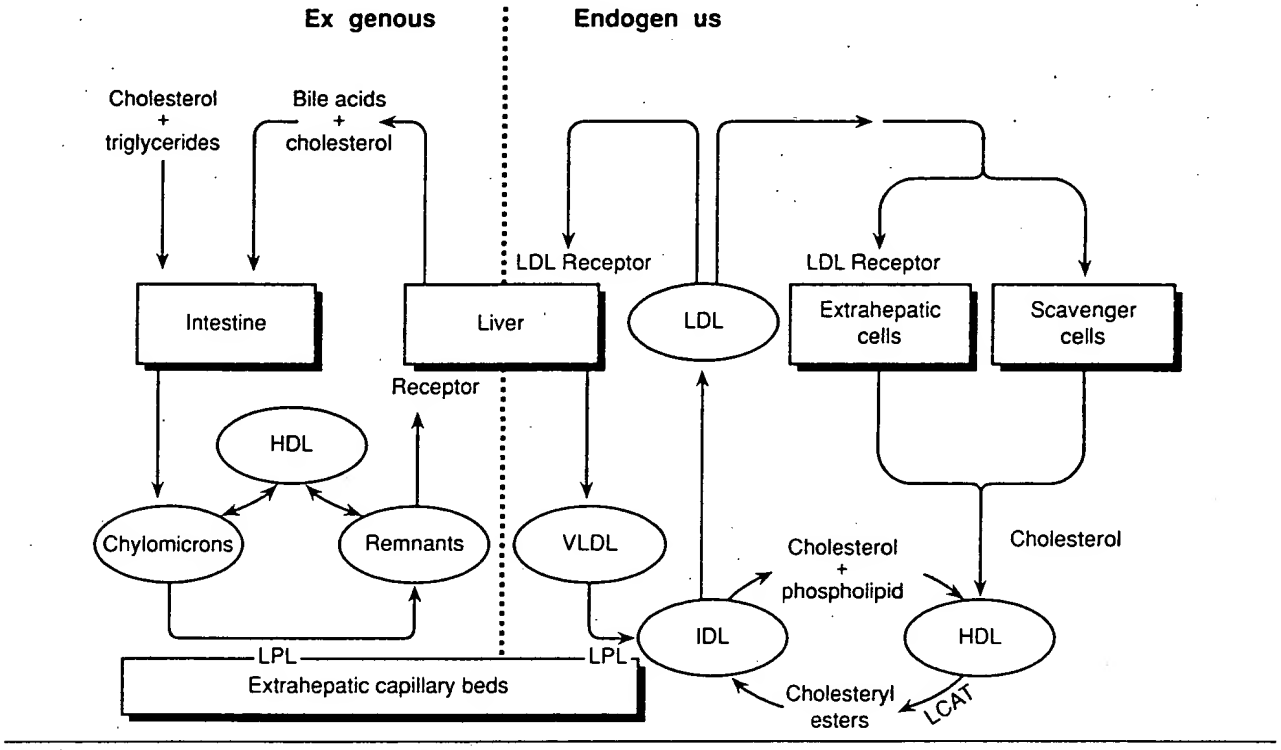

Fig. 1. Lipoprotein transport in humans, illustrating the exogenous and endogenous cholesterol uptake pathways. Both cycles begin with the secretion of triglyceride-rich particles [chylomicrons and very-low-density lipoprotein (VLDL)], which are converted to cholesterol-ester-rich particles [chylomicron remnants, intermediate-density lipoprotein (IDL), low density lipoprotein (LDL)]. The enzyme lecithin:cholesterol acyltransferase (LCAT) catalyzes the formation of cholesteryl esters. At least two lipoprotein receptors participate in these pathways [66]. (Reproduced from Brown et al. [1], with permission.)

Quantitation of Cholesterol Biosynthesis

Several methods have been used to estimate the rate of endogenous cholesterol biosynthesis, including HMG-Coa reductase or mevalonate assays, sterol balance techniques, and determination of the plasma kinetics of radioactive cholesterol or LDL. Studies evaluating the correlation between HMG-CoA reductase levels and cholesterol biosynthesis in humans are just now being done [7] due to the difficulty in obtaining appropriate tissue samples. Sterol balance techniques are onerous because of the long observation periods and exquisite dietary control of cholesterol intake that are required [8].

Methods incorporating radiolabeled cholesterol precursors are most commonly used to quantitate cholesterol biosynthesis. The rate of substrate incorporation is measured in tissue after in vitro incubation with the substrate or by injection of the substrate followed by measurement [9]. Assays of the rate of incorporation of [^{14}C]substrates into cholesterol are useful only for determining the relative change in rates of cholesterol synthesis [2]. More recently, the use of radiolabeled water to measure cholesterol synthesis was shown to be superior to [^{14}C]substrates [8,10,11] This technique has demonstrated that cholesterol synthesis in extrahepatic tissues is 5–20 times higher than previously thought, confirming that extrahepatic organs are self-sufficient with regard to cholesterol synthesis [2].

Cholesterol Biosynthetic Pathway

The initial step in cholesterol biosynthesis is the formation of acetoacetyl coenzyme A (CoA) from 2 moles of acetyl CoA. Condensation of a third mole of acetyl CoA results in the formation of the key intermediate, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) (Figure 2). The formation of HMG-CoA by the enzyme HMG-CoA synthase is one of several steps in the biosynthetic pathway that is regulated by feedback inhibition. HMG-CoA undergoes reduction by HMG-CoA reductase to form mevalonate in the most highly regulated step in the pathway [12–14]. It is this step that is inhibited by a new class of cholesterol-lowering drugs, the HMG-CoA reductase inhibitors (see be-

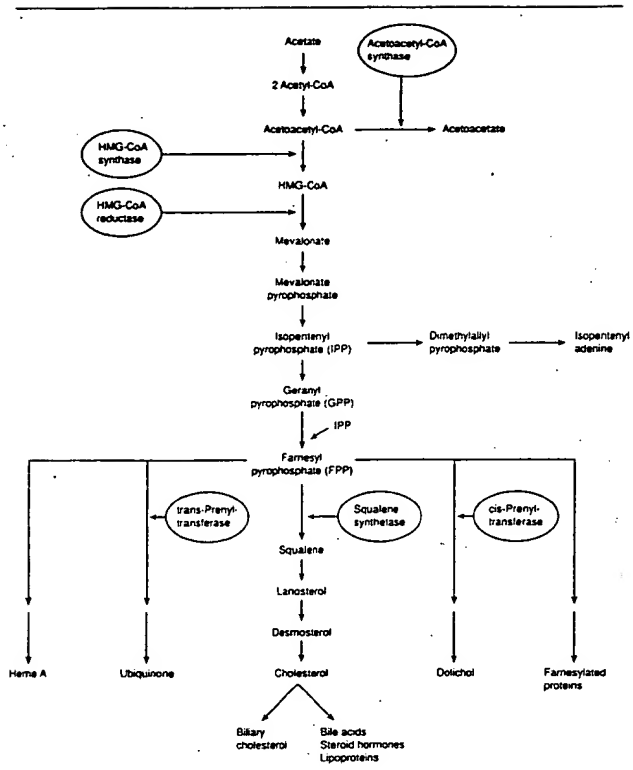

Fig. 2. Isoprenoid biosynthetic pathway. (Adapted from Edwards and Fogelman [12], Goldstein and Brown [13], and Panini et al. [14], with permission.)

low). Isopentenyl pyrophosphate (IPP), the 5-carbon structure that serves as the basic unit for isoprenoid (C_5H_8) synthesis, is formed from mevalonate through a series of phosphorylations and a decarboxylation. From IPP, isopentenyl adenine is formed, a compound that may play a role in regulating DNA replication [15]. IPP also polymerizes to form geranyl pyrophosphate (GPP), a 10-carbon isoprenoid from which the 15-carbon farnesyl pyrophosphate (FPP) is subsequently formed. With the formation of FPP, the isoprenoid pathway branches into multiple limbs, leading to the synthesis of both nonsterol and sterol products (Figure 2).

A series of condensations of FPP leads to the formation of either *trans*- or *cis*-polyprenyl pyrophosphates, from which nonsterol polyisoprene compounds, such as ubiquinones or dolichols, are formed. Ubiquinones participate in electron transport, and dolichols participate in glycoprotein synthesis. FPP and the 20-carbon isoprenoid geranylgeranyl pyrophosphate also serve as isoprenoid donors in newly discovered post-translational modifications of certain key proteins involved in cellular growth control and intracellular signalling (see below).

Condensation of 2 moles of FPP result in the formation of squalene, a 30-carbon molecule and the first dedicated step in sterol synthesis. The linear squalene molecule then undergoes a complex cyclization reaction and several intramolecular rearrangements to form the four-ring cyclopentanophenanthrene nucleus [16]. Subsequent demethylation steps yield the 27-carbon cholesterol molecule. The multiple reactions leading to the synthesis of cholesterol occur in both the cytosol and membrane compartments of the cell [17]. Water-soluble cholesterol precursors are thought to diffuse from membrane to membrane in the cytoplasm; squalene is the first membrane-bound cholesterol precursor. The rate of sterol synthesis is generally several hundred times greater than that of nonsterol synthesis, although the relative amount of isoprenoid diverted into the two pathways can be varied depending on cellular requirements [18].

The fate of newly synthesized cholesterol or that obtained by receptor-mediated uptake (see below) is varied; cholesterol may be catabolized to end products (e.g., bile acids, steroid hormones); it may be excreted directly in the bile; it may be stored as cholesteryl esters; or used in the biosynthesis of new membranes in dividing cells. The primary routes of cholesterol elimination are excretion in the feces as neutral or acidic (bile acid) sterols. The desquamation of the hair or skin and the conversion of cholesterol to steroid hormones account for smaller proportions of cholesterol elimination [3].

Bile acid synthesis accounts for the largest percentage of daily hepatic cholesterol catabolism. Bile acids are formed from cholesterol by the actions of more than 10 enzymes in the liver [19]. The first and rate-limiting step in the synthesis of bile acids is catalyzed by the microsomal cytochrome P-450 enzyme, cholesterol 7α-hydroxylase. The activity of this enzyme is regulated at the mRNA level by the flux of cholesterol through the liver and by the return of bile acids via the enterohepatic circulation [20]. Mechanisms of regulatory crosstalk between the pathways of cholesterol biosynthesis, uptake, and bile acid synthesis are currently under intense investigation (see below).

Cholesterol Synthesis and Cell Metabolism

Cholesterol and its precursors are essential elements for eukaryotic cell growth. Cholesterol is required as a building block for membranes of the nucleus, mitochondria, and microsomes. Cholesterol itself has little effect on DNA replication, however, it is required indirectly for cell division. As reviewed by Siperstein [4], inhibition of cholesterol synthesis with HMG-CoA reductase inhibitors or oxygenated sterols blocks cell growth and replication in a single cycle; cells cannot enter the DNA synthetic (S) phase. The addition of cholesterol and mevalonate reverses this inhibition and promotes cell growth and proliferation.

The requirement for cholesterol probably reflects the need to approximately double cell membrane mass

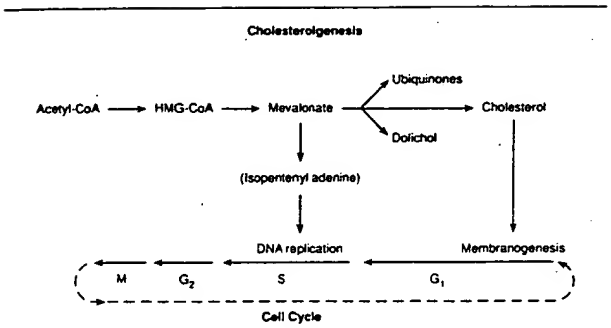

Fig. 3. The role of cholesterol biosynthesis in the cell cycle and DNA replication. Abbreviations as in text. (Reproduced from Siperstein [4], with permission.)

during division, whereas evidence suggests that mevalonate or a product derived from mevalonate plays an important role in the initiation of DNA replication. This relationship was detected initially by the threefold rise in HMG-CoA reductase activity observed at or immediately prior to S-phase DNA replication [21]. Preliminary studies using double thymidine blocked baby-hamster kidney (BHK) cell indicated that stimulation of DNA synthesis may be mediated through isopentenyl adenine; this compound was 100 times more active than mevalonate in reversing the suppression of DNA synthesis by HMG-CoA reductase inhibitors [15]. Subsequent studies have shown, however, that the effects of mevalonate byproducts on DNA replication and cell proliferation may be quite complex [22]. The interrelationships between the cell replication cycle, DNA synthesis, and cholesterologenesis are illustrated in Figure 3 [4].

An exciting development in the area of cholesterol synthesis and cell growth has been the discovery that multiple proteins are post-translationally modified by the covalent attachment of isoprenoids to cysteine residues [13]. These proteins include the nuclear lamins, which play a fundamental role is assembly of the nucleus [23], an ubiquitous class of small (~20,000 M_r) GTP-binding proteins [24] that include oncogene products such as *ras* [25], and a subunit of certain trimeric intracellular signalling proteins (the so-called G proteins) that mediate the production of multiple second messengers within the cell [26].

The clinical implications of these findings have become more apparent as premalignant and malignant cells may lack certain regulatory mechanisms for negative feedback control of cholesterol metabolism [4]. Thus, these cells demonstrate high levels of mevalonate and cholesterol synthesis, which normally would decrease in response to excess cholesterol. Some investigations have linked the source of this defect to HMG-CoA reductase, which displays a higher state of activation in malignant cell lines as compared with normal, nonmalignant cells [4]. The increased activity of HMG-CoA reductase and the finding that certain cancer-causing genes such as *ras* are modified by the addition of isoprenoids has led to the suggestion that HMG-CoA reductase inhibitors may have some utility as antineoplastics [27,28]. Alternatively, enzymes that isoprenylate proteins may serve as more focused therapeutic targets [13].

Regulation of Cholesterol Homeostasis

Cholesterol homeostasis is regulated and maintained by three interrelated feedback mechanisms: regulation of LDL receptor production, activity and regulation of HMG-CoA reductase and other enzymes in the biosynthetic pathway, and regulation of cholesterol 7α-hydroxylase in bile acid synthesis.

LDL receptor regulation

As discussed above, cells have two means to satisfy their cholesterol requirements, either via uptake of cholesterol-carrying lipoprotein particles mediated by the LDL receptor (Figure 1) or by synthesis from acetate precursors (Figure 2). Cells that use little cholesterol, such as those of the kidney or skeletal muscle, can satisfy their requirements by endogenous cholesterol synthesis and thus tend to have low numbers of LDL receptors. Cells of the liver and adrenal glands, however, because of their enhanced cholesterol requirements, contain large numbers of LDL receptors [2,29] and actively synthesize cholesterol.

Because of its central role in cholesterol metabolism, the cell biology, biochemistry, molecular biology, and genetics of the LDL receptor pathway have been extensively investigated [30]. These studies have revealed that the human LDL receptor is a cell-surface glycoprotein [31] with high affinity for several classes of cholesterol-carrying lipoproteins that contain apolipoprotein B and apolipoprotein E [32]. The LDL receptor is estimated to account for the uptake and degradation of 65–80% of LDL in humans [2]. This clearance is accomplished by receptor-mediated endocytosis (Figure 4), a process involving an initial binding event at the cell surface followed by a multistep invagination and intracellular trafficking itinerary. Within the cell, bound LDL dissociates from the receptor and is transported to the lysosome for breakdown. After ligand release, the LDL receptor recycles to the cell surface, where it undergoes additional rounds of binding and internalization. The cycle requires about 10 minutes to complete; thus in a typical cell an LDL receptor may participate in hundreds of internalization events [33].

Cholesterol that is released from LDL during breakdown in the lysosome plays two important regulatory roles. First, it activates the acyl cholesterol acyl transferase (ACAT) enzyme, which catalyzes the formation of readily stored cholesteryl esters [30]. Second, excess cholesterol or a derivative brings about a diminution of LDL receptor gene transcrip-

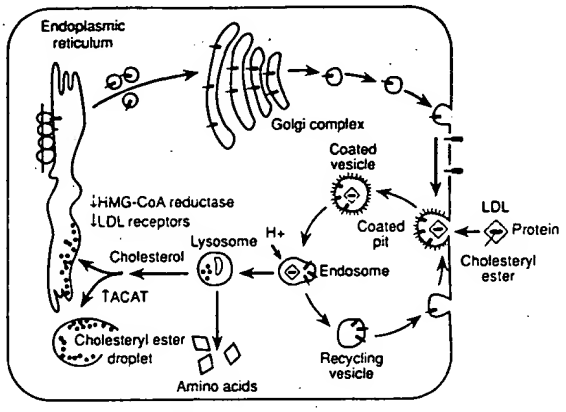

Fig. 4. The LDL receptor pathway of cholesterol uptake. Abbreviations as in text. (Reproduced from Brown and Goldstein [30], with permission.)

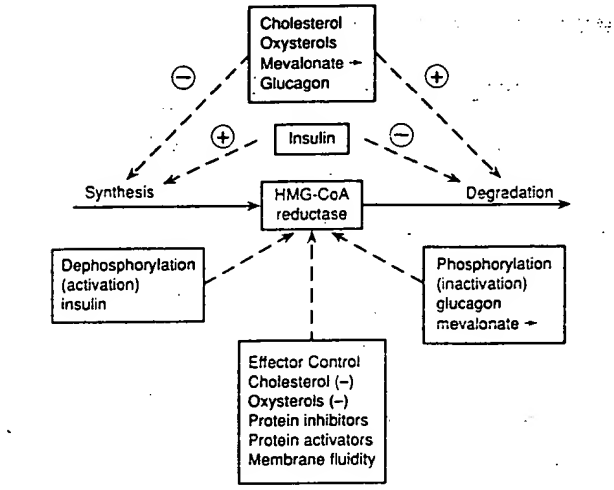

Fig. 5. Positive (+) and negative (–) control of hepatic HMG-CoA reductase by long-term (upper half) and short-term (lower half) mechanisms. "Mevalonate→" signifies that products of mevalonate are involved in the direct regulation of HMG-CoA reductase rather than mevalonate itself. (Reproduced from Gibson [39], with permission.)

tion in the nucleus [34], thus reducing the synthesis of new LDL receptors. Through these regulatory actions, the cell protects itself from a buildup of excess cholesterol. Conversely, when a cell such as a hepatocyte in the liver is starved for cholesterol or experiences an unusual demand for the molecule (e.g., to replace bile acids sequestered by the administration of bile-acid binding resins), cholesteryl ester pools are metabolized and LDL receptor synthesis is increased [30]. LDL receptor synthesis is also enhanced when cholesterol synthesis is suppressed, such as during the administration of HMG-CoA reductase inhibitors [35].

HMG-CoA reductase regulation

HMG-CoA reductase is a membrane-bound enzyme of the endoplasmic reticulum and is one of the most highly regulated enzymes in nature [13]. The human protein contains 888 amino acids that can be divided into a membrane-associated domain of 339 residues and a soluble catalytic domain of 548 residues [36,37]. Studies of the sequence of HMG-CoA reductase cDNAs derived from various animal species suggest that the structure of both the membrane-associated and catalytic domains of HMG-CoA reductase are highly conserved [38].

That HMG-CoA reductase is a central control point of cholesterol synthesis is indicated by the multiple levels at which the enzyme is regulated [39] (Figure 5). Thus, synthesis of HMG-CoA reductase mRNA [40], translation of the mRNA [41,42], degradation of the protein [43], and activity of the enzyme itself [44] are regulated in response to cellular cholesterol and isoprenoid demand. In addition, HMG-CoA reductase is subject to a diurnal cycle regulation [45] and to complex hormonal influences [46].

Cholesterol and mevalonate individually suppress the synthesis of HMG-CoA reductase. However, maximal suppression of the enzyme requires the presence of both cholesterol and a nonsterol metabolite of mevalonate [13]. Sterols decrease the transcription of the gene [40] and increase the degradation of the protein via the membrane-associated domain [43]. The nonsterol mevalonate-derived product decreases translation of the mRNA and also increases degradation of the protein [41]. When cellular sterol concentrations decrease, the multilevel regulatory process can increase HMG-CoA reductase synthesis by 200-fold. This dramatic response is accomplished by inducing transcription of the gene and by slowing the rate of HMG-CoA reductase degradation [13]. When cholesterol is present in adequate amounts, both HMG-CoA synthase [the enzyme preceding reductase in the synthetic pathway (Figure 2)] and HMG-CoA reductase activities can decline by 90%, with cells using the remaining 10% of mevalonate production solely for the production of nonsterol products [47].

HMG-CoA reductase activity may also be regulated by a number of other mechanisms, including conversion of the enzyme between active and inactive forms by way of a phosphorylation (inactive form)-dephosphorylation (active form) event dependent on protein kinases [44], by changes in the fluidity of the endoplasmic reticulum membrane brought about by increasing (less fluid) or decreasing (more fluid) cholesterol levels in the organelle [18,39], and finally by isoprenylated proteins. Because mevalonate is required for isoprenylation, decreased mevalonate production will result in an accumulation of unmodified proteins. These proteins may have a role in upregulating or downregulating translation or degradation of HMG-CoA reductase [13].

The mechanisms by which the translation of the HMG-CoA reductase mRNA and degradation of the protein are regulated remain obscure. However, substantial insight has been gained into the mechanisms of transcriptional regulation of the HMG-CoA reductase gene and other genes encoding enzymes of cholesterol synthesis, as well as the LDL receptor gene.

The 5′-flanking regions of the HMG-CoA reductase, HMG-CoA synthase, and LDL receptor genes contain one or more copies of an eight-nucleotide DNA sequence termed the *sterol regulatory element* or *SRE* [48,49]. The SRE element can act to stimulate the expression of a gene in the absence of sterols, as it does in the LDL receptor gene, or the SRE can bring about a decrease in mRNA production in the presence of sterols, as it does in the HMG-CoA reductase gene [13]. Given the presence of SREs in these multiple genes, and the observation that many enzymes in the cholesterol synthesis pathway are subject to sterol-mediated feedback regulation [50,51], it is conceivable that SREs will be found in the 5′-flanking regions of multiple genes involved in this pathway. Should this hypothesis prove correct, the SRE and proteins that interact with this element may represent targets for drugs that could increase LDL receptor expression (thus decreasing serum cholesterol levels) or that could suppress multiple genes involved in cholesterol synthesis.

Cholesterol 7α-hydroxylase regulation

The production of bile acids in the liver represents the major catabolic pathway for cholesterol disposal. To ensure homeostasis, the amount of cholesterol diverted into bile-acid synthesis is tightly regulated by modulating the activity of cholesterol 7α-hydroxylase, the first enzyme in the pathway. Although early studies demonstrated the importance of this enzyme [52], insight into the mechanisms by which activity is regulated had to await the purification of the enzyme and the cloning of the mRNA [53]. Subsequent studies have revealed a tissue-specific (liver only) expression of the cholesterol 7α-hydroxylase gene [20], induction of the gene by dietary cholesterol and suppression by bile acids [20,54,55], and diurnal regulation [56–58]. In addition, induction by bile-acid binding resins and complex hormonal regulation has been demonstrated. It is clear that the regulation of cholesterol 7α-hydroxylase, and hence cholesterol catabolism, occurs at multiple levels and may rival that of HMG-CoA reductase in its complexity.

Precisely how the pathways of cholesterol supply and catabolism are coordinately or noncoordinately regulated is currently an area of intense research interest. This interest arises in part from the observation that increased bile-acid production represents a mechanism for decreasing serum cholesterol. Bile-acid binding resins (cholestid, cholestyramine) are a case in point. These agents prevent the return of bile acids to the liver and thus force the liver to synthesize more bile acids to maintain a constant pool of these essential molecules. The resulting increased demand for cholesterol is met by increasing LDL receptor-mediated uptake of lipoprotein particles, leading to a decrease in serum cholesterol. In the future it may be possible to develop drugs that directly stimulate the cholesterol 7α-hydroxylase gene [59] in order to decrease serum cholesterol levels.

HMG-CoA reductase inhibitors

The catalyst for the discovery of much of what is known regarding the regulation of cholesterol synthesis was the isolation and characterization of competitive inhibitors of HMG-CoA reductase [60]. These compounds were used to reveal the regulation of the pathway by nonsterol mevalonate-derived products [47], to clone the HMG-CoA reductase gene [61], to implicate a role for cholesterol and its biosynthetic intermediates in DNA synthesis [4], and to gain insight into the role of isoprenylated proteins in cellular regulation and cholesterol metabolism [13].

The development of the HMG-CoA reductase inhibitors had a tremendous impact on the clinical management of persons with elevated serum cholesterol levels, a major risk factor for the development of coronary heart disease. The discovery and use of therapeutic inhibitors of HMG-CoA reductase (e.g., lovastatin, pravastatin, simvastatin) have allowed rapid and sustained reductions of cholesterol levels of up to 40% when used alone, and even higher (>50%) when used in combination with other lipid-lowering agents [62]. Not only do these drugs lower serum cholesterol, but emerging data also suggest that this reduction can, in some individuals, promote atherosclerotic lesion regression [63].

Previous attempts to inhibit cholesterol synthesis with agents (e.g., triparanol) that interrupted the biosynthetic pathway at a later stage (conversion of desmosterol to cholesterol) were unsuccessful because they allowed the accumulation of toxic metabolites that were associated with complications (lenticular cataracts, ichthyosis) [64,65]. The HMG-CoA reductase inhibitors, in contrast, inhibit the cholesterol biosynthetic pathway at an earlier stage, when cholesterol precursors are water soluble (Figure 2) and are able to be metabolized via other biosynthetic pathways.

Conclusions

Cholesterol is a crucial molecule for eukaryotic cell growth and development. The body has at its disposal a variety of means to regulate the supply and breakdown of this essential molecule. Over the last two decades, research has provided substantial insight into the complex regulation of cholesterol homeostasis and has provided the clinician with powerful new drugs for the treatment of hypercholesterolemia. As we

progress further in our understanding of cholesterol and its regulation, it is likely that still more powerful therapeutics (e.g., site-specific cholesterol inhibitors) will be discovered for use in the treatment of the many diseases with a cholesterol-linked etiology.

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